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Species

Thelohanellus filli sp.n., a pathogenic myxosporean infecting gills of cultured carp, *Labeo rohita* (Hamilton 1822) in Punjab, India

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ABSTRACT

The plasmodia of *Thelohanellus filli* sp. n. were found infecting gills of *Labeo rohita* (Hamilton, 1822). The infection rate was 35% (20 fishes were examined and 7 fishes were infected) in four cultured farm in district Pathankot, Punjab. Fishes were 8-10 months old and 15 - 20% fish mortality was recorded in four farms located in the rural area adjoining the district Pathankot, Punjab. Plasmodia measure 0.8-1.5x1.5-2mm in size filled with 600-1000 spores were detected in the gill filament. Spores histozoic, large, measure 27.08x10.56µm in size, elongate pyriform in valvular view, tapering anteriorly with bluntly pointed anterior end and rounded posterior end. Shell valves thick, smooth, symmetrical measure 1.30µm in thickness. Parietal folds absent. Polar capsule pyriform in shape measure 16.25x8.25µm, with bottle neck shaped anterior end and rounded posterior end. Polar capsule is situated anteriorly and occupies more than half of the spore body cavity. Polar filament form 12-14 coils arranged perpendicular to the polar capsule axis and thick, thread-like measuring 89.85µm in length when extruded. Sutural line straight and distinct. Sporoplasm homogenous, granular occupies whole of the extracapsular space behind the polar capsule. An iodinophilous vacuole present, measure 4.08µm in diameter. Two sporoplasmic nuclei present measure 2.5-2.9 µm in diameter.

Keywords: aquaculture, histopathology, gills, Indian major carp, *Labeo rohita*, *Thelohanellus*

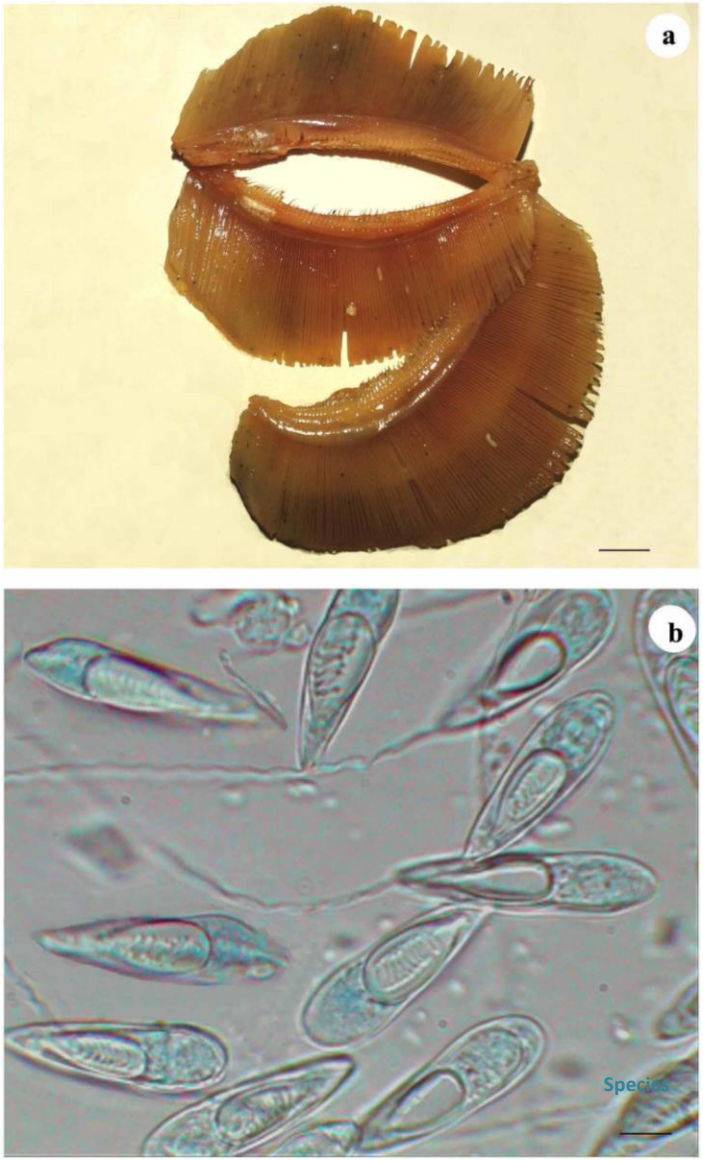

Figure 1

(a) Showing cysts (plasmodium) on the gills of *Labeo rohita* infected with *Thelohanellus filli* sp. n.

(b) Showing fresh spores of *T. filli* sp. n.

Scale bar=10 µm

1. INTRODUCTION

Fisheries have always played a pivotal role in food and nutrition all over the world. Aquaculture of major carps has increased in India during recent years. Myxozoan parasites are economically important as they infect fish harvested for food. These parasites infect fresh water fishes as well marine fishes. Large number of myxozoan parasites infecting fishes in wetlands of Punjab have been recorded (Kaur and Singh 2008, 2008/2009, 2009, 2010a, 2010b, 2010/2011, 2011a,b,c,d,e,f, 2012a,b; Singh and Kaur 2012, 2013) causing serious threat to fish health. Among myxozoan parasites, *Thelohanellus* is a small genus with a total of 108 nominal species worldwide and 40 species from India (Zhang et al. 2013). Myxozoan parsites are mostly histozoic and coelozoic. The presence of plasmodia was revealed with whitish cysts with microscopic spores. Large cysts were easily spotted with naked eye. But minute cysts were detectable under the microsope with the help of histological sections. During the present study a new myxosporean parasite, *Thelohanellus filli* was collected from cultured carp, *Labeo rohita* from Pathankot (Punjab) India and described morphologically and morphometrically.

2. MATERIALS AND METHODS

Fishes from four fish farms located in rural area of district Pathankot, Punjab were brought in live condition to the laboratory for further investigation. Fishes ranged 25–30cm in length and were 8-10 months old. The selected pond was a polyculture having *Labeo rohita* Hamilton, *Catla calta* Hamilton, *Cirrhinus mrigala* Hamilton, *Ctenopharyngodon idellus* Valenciennes and *Cyprinus carpio* Linnaeus. The temperature of the pond water at the time of the collection was 30-32˚C. The organs such as gills, gut, eyes, fins, scales and skin were examined for infection. Infected organs were fixed in Bouin's fixative for histological studies.

Each plasmodium was ruptured in normal saline (0.85%) with the help of a needle on a clean slide and examined under light microscope for the presence of spores. Fresh spores were studied in Lugol's iodine solution to confirm the presence of iodinophilous vacuole.

To make dry preparations thin smears were air dried, fixed in methanol and stained with Giemsa. In the case of permanent (wet) preparations, smears on clean slides were fixed in Schaudinn's and Bouin's fixatives. The stains such as Heidenhain's Iron-haematoxylin and modified Ziehl-Neelsen were used to study the detailed spore morphology (Figure 2a, b).

2.1. Histopathology

Infected gills were cut into small pieces and fixed in Bouin's fixative. Tissue samples were dehydrated in ascending grades of ethanol, cleared in xylene, embedded in paraffin wax, sectioned at 7-8µm thickness and stained with Luna's staining method. In Luna's method (Luna, 1968), the cysts stained bright red and rest of the gill tissue stained blue in color. This technique demonstrated the location of cysts within the gills and therefore can be useful in histological diagnosis of the myxosporean parasites (Figure 4).

3. RESULTS AND DISCUSSION

3.1. Description of plasmodium of *Thelohanellus filli* sp. n. (Tables: 1, 2)

Large, creamish white, oval, present on the gill filament, 3-4 in number, measure 0.8-1.5x1.5-2mm in size, 600-1000 spores per plasmodium (Figure 1b).

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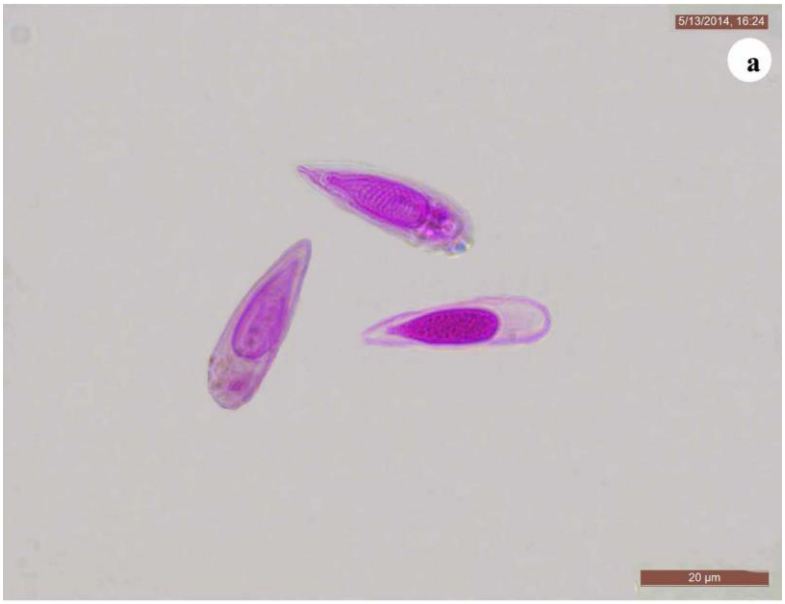

Figure 2

(a) Spores stained in Ziehl-Neelsen

(b) Spores stained in Iron Haematoxylin

Scale bar=20µm

3.2. Description of spore

(Measurements based on 10-12 spores in frontal view); (Figure 3)

The spores are histozoic, large, measure 27.08x10.56µm, elongate pyriform in valvular view, tapering anteriorly with bluntly pointed anterior end and broad rounded at the posterior end. Shell valves thick, smooth, symmetrical measuring 1.30µm in thickness with straight and distinct sutural line. Parietal folds are absent. Polar capsule is pyriform in shape measure 16.25x8.25µm, with bifurcated anterior end and rounded posterior end. Polar capsule is situated anteriorly and occupies more than half of the spore body cavity. Polar filament form 12-14 coils arranged perpendicular to the polar capsule axis and is thick, thread-like measure 89.85µm in length when extruded. Sutural line is straight and distinct. Sporoplasm homogenous, granular occupies whole of the extracapsular space behind the polar capsule. An iodinophilous vacuole is present, measure 4.08µm in diameter. Two sporoplasmic nuclei present measure 2.5-2.9µm in diameter.

3.3. Taxonomic summary of *T. filli* sp.n.

Host : *Labeo rohita* (Ham.) vern. rohu

Locality : Fish farms, Pathankot

Site of infection : Gill filament (intrafilamental)

Prevalence of infection : (35%) 7/20

Clinical Symptomatology : Whitish cysts, haemorrhagic and mucus laden gills

Etymology : The specific epithet *filli* has been given on the basis of filiform polar filament which instantly everted at the time of rupturing of plasmodium

3.4. Histopathological studies

The gross morphology of infected gills was minute 2-3 whitish cysts on the gill filaments (Figure 1a). The plasmodia appeared ellipitical to ovoid in shape. Histologically, each plasmodium occupied the entire gill filament bounded by thin cellular layer (CL). The plasmodium damaged more than 50% of the gill filament and gill lamellae. Considerable amount of cellular debris was detected intermixed within the spores inside the plasmodia. Intrafilamental location of plasmodium resulted in complete distortion of the gill cells.

3.5. Differential Diagnosis

The present species, *Thelohanellus filli* sp. n. was compared with *T. rohitae* Southwell and Prashad (1918) from gills of *Labeo bata* and *L. rohita*; *T. gangeticus* Tripathi (1952) from muscles of *Chela bacaila*; *T. andhrae* Qadri (1962) from gills of *Labeo fimbriatus*; *T. jiroveci* Kundu and Haldar (1981) from branchiae of *L. bata*; *T. rodgii*, Hagargi, Kundu and Haldar (1979) from gills of *Labeo calbasu*, *T. disporomorphus* Basu, Modak and Haldar (2006) from tail fins of *Cirrhinus mrigala*, *T. valeti* Fomena and Boux (1987) from gill arches and stomach of *Barbus jae Boulenger*; *T. chilkensis* Kalavati and Vaidehi (1991) from gall bladder of *L. rohita*; *T. bifurcata* Basu and Haldar (1999) from gill lamellae of *L. rohita* x *Catla catla; T. endodermitus* Mukhopadhay and Haldar (2004) from under scales of *Labeo rohita*; *T. anilae*, Hemananda, Bandhopadhay, Mohilal and Mitra (2010) from gills of *Labeo rohita*; *T. testudineus* Liu, Jia, Huang and Gu

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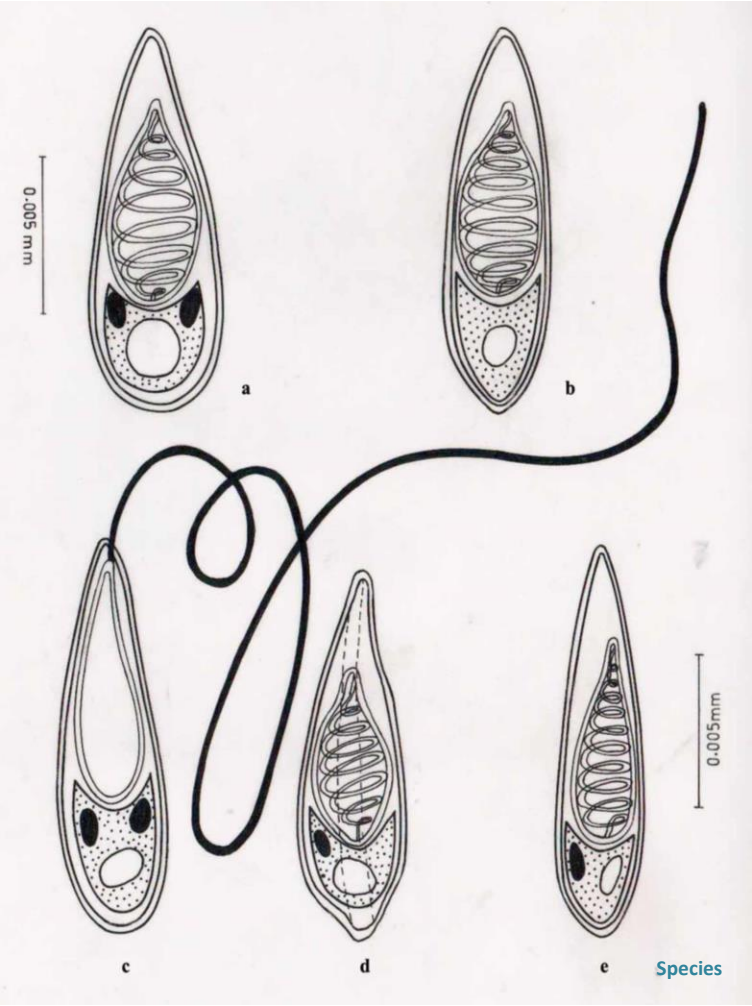

Figure 3

Line drawing (Camera Lucida) of spores of *T. filli* sp. n.
(a, b) Spore stained in Ziehl-Neelsen (valvular view),
(c) Spore stained in Iron Haematoxylin (polar filament extruded)
(d) Fresh spore (sutural view); (e) side view
Scale bar=0.005mm

(2014) from skin of *Carassius auratus gibelio* but differ from all of the above species in morphological and morphometric characterstics.

The spores of present species are pyriform with bluntly pointed anterior end and rounded posterior end, in this respect, the present species is comparable with the spores of *T. bifurcata*; *T. chilkensis* and *T. rohitae*. The shape of the present species (LS/WS: 2.56) also resemble with spores of *T. chilkensis* in which the inner wall of the shell have a distinct constriction formed by 2 indentations on either side at the posterior one third. However, the present species differ from *T. bifurcata* (LS/WS: 3.7) in having much wider spores (LS/WS: 2.56) and from *T. chilkensis* (LS/WS: 2.7) due to the absence of any constriction at posterior end in the present species (Table 1). The present species is differentiated from *T. rohitae* in having elongate pyriform with acutely pointed anterior end and rounded posterior end.

In addition, the anterior end of the polar capsule in *T. bifurcata* is elongately pyriform, bifurcated and occupy more than two third of the spore body cavity; elongate pyriform with acutely pointed anterior end and rounded posterior end in *T. rohitae,* unlike in the present species in which it is broadly pyriform with bluntly pointed anterior end occupying more than half of spore body cavity. *T. rodgii* is differentiated from the present species in having pointed anterior end as in present species anterior end is bluntly pointed. The present species also differ from *T. chilkensis* in which the anterior end of the spore is narrow, flat at the tip and contain a flask shaped polar capsule with a small neck opening 2-3µm below the anterior tip. The present species is differentiated from *T. gangeticus*; *T. jiroveci*; *T. andhrae*; *T. endodermitus*; *T. valeti*; *T. anilae* and *T. testudineus* by larger spore size and absence of micro and macrospore in the present species. In *T. anilae*, shape of the polar capsule is large elongate and tear shaped but in the present species shape of the polar capsule is pyriform and having bottle neck shaped anterior end. The polar capsule of the present species occupied more than half of the spore cavity; this character differentiated it from *T. gangeticus*; *T. jiroveci*; *T. andhrae*; *T. endodermitus*; *T. valeti* and *T. testudineus* in which polar capsule occupied less than half of the spore cavity. *T. disporomorphus* is different from present species in having larger and broader size (LS/WS: 3.6), and also by host and tissue specificity as *T. disporomorphus* is infecting tail fin of *C. mrigala* in comparison to the present species which infects the gills of *Labeo rohita*.

In view of the above differences, the present species under study is proposed as new to the science and named as *T. filli* sp. n.

3.6. Histopathology

Histologically, the present species, *T.filli* sp. n. is located within the gill filament. Due to the presence of spores within the entire length of the gill filament, the blood vessels were dilated and bounding endothelial cells were highly compressed. Similar observations were made by Molnar (2002); Molnar and Szekely (1999); Awal et al., (2009); Chavda et al. (2010) infecting capillary network of the gill lamellae and respiratory plate. Also, Rukyani (1990), Azevedo et al. (2010) and Campos et al. (2011), Raissy and Ansari (2011) reported the alterations in capillary network, hyperplasia of gill epithelium and structural disorganization of secondary lamellae. In present study, *T. filli* sp.n. distorted the respiratory surface due to the development of plasmodia on the gill filament, similar observation have also been made by MacCraren et al. (1975) in gill infections of American catfish with *Henneguya exilis*, Kalavati and Narasimhamuri (1985) in *Channa punctata* with *Henneguya waltairensis* and Rukyani (1990) in carp with *Myxobolus koi*. According to Adriano et al. (2009) these alterations may partially compromise gill functions and therefore

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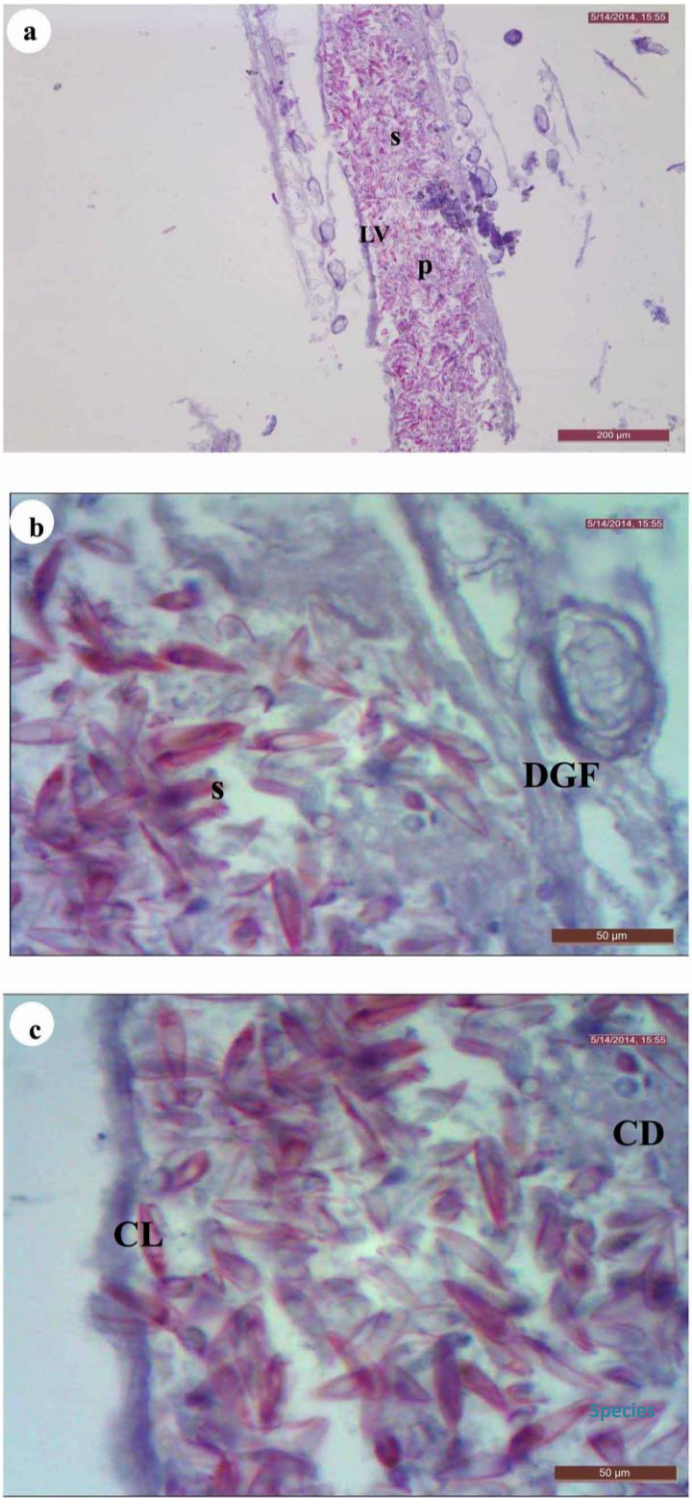

Figure 4

Histopathology of gills of *Labeo rohita* infected with *T. filli* sp. n
(a) Sagittal section of gills of *Labeo rohita* showing intrafilamental vascular type (LV) plasmodia (P) infected with *T.filli* sp. n. (100x) scale bar= 200 µm
(b) Magnified view of infected gill showing deformed gill filament (DGF) forming cyst enclosing numerous spores (S) (400x)
(c) Sagittal section of infected gills of *Labeo rohita* showing cellular layer of plasmodia (CL); cellular debris (CD).
LUNA'S METHOD; Scale bar (b, c)=50 µm

diminish the respiratory capacity and ionic exchange. Schulman (1957) and Kalavati and Narashimhamurti (1985) reported that rupture of cysts leads to the haemorrhagic gills resulting in the loss of blood.

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Table 1

Measurements (µm) and ratio of *Thelohanellus filli* sp. n.

Characters	Range	Mean Values	SD
LS	25.66-28.5	27.08	2.13
WS	10.12-11.0	10.56	0.55
LPC	16.26-17.0	16.63	2.03
WPC	8-8.5	8.25	0.81
Ratio: LS/WS		2.56	
NC		12-14	
Parietal Folds		Absent	

Table 2

Comparative description of *Thelohanellus filli* sp. n. with morphologically similar species (measurements are in micrometer)

Species	Host	Site of infection	Locality	Spore	Polar capsule
***Thelohanellus filli* sp. n. present study**	*Labeo rohita*	Gills (intrafilamental)	Pathankot, Punjab (India)	27.08x10.56	16.63x8.25
***T. rohitae* Southwell and Prashad, 1918**	*L. rohita* and *L. bata*	Gills	West Bengal(India)	31.5x11.5	18x15.9
***T. gangeticus* Tripathi, 1952**	*Chela bacaila*	Muscles	West Bengal (India)	16.85-5.4	7.2x2.5
***T. andhrae* Qadri, 1962**	*L. fimbriatus*	Gills	Andra Pradesh (India)	12.85x5.0	7.0x2.25
***T. rodgii* Hargarfi *et al.*, 1979**	*L. calbasu*	Gills	West Bengal (India)	36.0x12.5	17.5x7.5
***T. jiroveci* Kundu and Haldar, 1981**	*L. bata*	Branchiae	Ranaghat, West Bengal (India)	16.3x6.8	7.3x4.1
***T. chilkensis* Kalavati and Vaidehi, 1991**	*L. rohita*	Gall bladder	Odisha	26.7x8.7	17.5x7.01
***T. valeti* Fomena and Bouix, 1987**	*Barbus aspilus*	Operculum and Stomach wall	Africa	12.0x4.7	6.25x2.3
***T. bifurcata* Basu and Haldar, 1999**	*L. rohita* x*Catla catla*	Gill lamellae	West Bengal (India)	34.89x9.21	23.3x6.6
***T. endodermitus* Mukhopadhyay and Haldar, 2004**	*L. rohita*	Under surface of scales	West Bengal (India)	13.66x5.35	7.14x3.0
***T. disporomorphus* Basu and Haldar, 2006**	*C. mrigala*	Tail fin	Halisahar West Bengal (India)	32.1 and 14.2x8.9 and 8.5	21.1 and 5.2x7.9 and 4.0
***T. anilae* Hemananda *et al.*, 2010**	*L. rohita*	Gills	Hingalganj West Bengal (India)	33.27 and 13.26x12.75 and 6.8	17.55 and 7.31x5.35 and 3.10
***T. testudineus* Liu *et al.*, 2014**	*Carassius auratus gibelio*	Skin	Huanggang, Hubei Province (China)	19.7x7.6	11.1x5.6

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